Article:

Barakzai, Safia, Wells, Jeremy orcid.org/0000-0002-4159-7179, Parkin, T. et al. (1 more author) (2018) Overground endoscopic findings and respiratory sound analysis in horses with recurrent laryngeal neuropathy after unilateral laser ventriculocordectomy. Equine Veterinary Journal. ISSN 2042-3306

https://doi.org/10.1111/evj.12993

Overground endoscopic findings and respiratory sound analysis in horses with recurrent laryngeal neuropathy after unilateral laser ventriculocordectomy

Barakzai S.Z.[1], Wells, J. [2], Parkin, T.D.H. [3], Cramp, P[4].
[1] Equine Surgical Referrals, Clapham, Sussex, UK.
[2] Department of Music, University of York, Yorkshire, UK
[3] Weipers Centre, University of Glasgow, Beardsden, UK.
[4]Hambleton Equine Clinic, Great Ayton, North Yorkshire, UK

Ethical considerations: Informed client consent was obtained for all horses included in this study

Competing interests: None

Sources of Funding: Horserace Betting Levy Board Small Project Grant

Acknowledgements: Dr Chris Baldwin for performing RGA ratio and LRQ measurements.

Authorship:
S. Z. Barakzai: study design, data collection and study execution, data analysis and interpretation, preparation of manuscript

J. Wells: Data analysis and interpretation, preparation of the manuscript

T. Parkin: Statistical analysis, preparation of manuscript

P. Cramp: study design, data analysis and interpretation, preparation of the manuscript

Overground endoscopic findings and respiratory sound analysis in horses with recurrent laryngeal neuropathy after unilateral laser ventriculocordectomy

Summary

- **Background:** Unilateral ventriculocordectomy (VeC) is frequently performed, yet objective studies in horses with naturally occurring RLN are few.
- **Objectives:** To evaluate respiratory noise and exercising over-ground endoscopy in horses with grade B and C laryngeal function, before and after unilateral laser VeC.
- **Study Design:** Prospective study in clinically affected client-owned horses.
- **Methods:** Exercising endoscopy was performed and concurrent respiratory noise was recorded. A left sided laser VeC was performed under standing sedation. Owners were asked to present the horse for re-examination 6-8 weeks post-operatively when exercising endoscopy and sound recordings were repeated. Exercising endoscopic findings were recorded, including the degree of arytenoid stability. Quantitative measurement of left-to-right quotient angle ratio (LRQ) and rima glottidis area ratio (RGA) were performed pre- and post- operatively. Sound analysis was performed, and measurements of the energy change in F1, F2 and F3 formants between pre- and post-operative recordings were made and statistically analysed.
- **Results:** Three grade B and 7 grade C horses were included. 6/7grade C horses pre-operatively had bilateral vocal fold collapse (VFC) and 5/7 had mild medial deviation of the right ary-epiglottic fold (MDAF). Right VFC and MDAF was still present in these horses post-operatively. Sound analysis showed significant reduction in energy in

formant F2 (P=0.05) after surgery. Ongoing left arytenoid instability, right VFC and MDAF caused continued noise.

- **Main Limitations:** The study sample size was small and multiple dynamic abnormalities made sound analysis challenging.
- **Conclusions:** RLN-affected horses have reduction of sound levels in F2 after unilateral laser VeC. Continuing noise may be caused by other ongoing forms of dynamic obstruction. Unilateral VeC is useful for grade B horses based on endoscopic images. In Grade C horses, bilateral VeC, right ary-epiglottic fold resection +/- laryngoplasty might be a better option than unilateral VeC.

Introduction:

To most owners, trainers, and referring veterinarians, reduction of respiratory noise is a key factor when determining whether or not an upper respiratory tract surgery has been 'successful'. Respiratory noise has shown an association with other objective measures of upper airway parameters, such as trans upper airway inspiratory pressures, and is therefore a useful measure of surgical success[2]. Unilateral ventriculocordectomy (VeC) is probably one of the most frequently performed upper respiratory tract surgeries, yet there are no publications that quantitatively analyse its effect on respiratory sound production in horses with naturally occurring recurrent laryngeal neuropathy (RLN), including in horses with grade B exercising laryngeal function[1] (Table 1) and vocal fold collapse. Two previous experimental studies have found that bilateral VeC (via laryngotomy) or unilateral transendoscopic laser VeC restore sound levels close to baseline (pre-neurectomy levels) in grade 4 horses[2,3] which is rather surprising, given that the left arytenoid is presumably still collapsing during inspiration, causing respiratory obstruction and turbulent airflow. A clinical study of draft horses with grade 4 laryngeal function[4] (left-sided hemiplegia) found that bilateral surgical VeC significantly reduced inspiratory noise, but these horses were only exercised at a trot[4].

Furthermore, no previous study has evaluated horses both pre- and post-VeC using exercising endoscopy, to ascertain if the surgery has resulted in stabilisation of the left arytenoid, or if other dynamic respiratory abnormalities are present. We aimed to evaluate exercising over-ground endoscopic videos (both subjectively and objectively) and respiratory sound production in horses before and after unilateral VeC, including horses with both grade B and grade C laryngeal function at exercise[1].

Materials and Methods:

This study was a prospective clinical trial. A power calculation using sound analysis data from previously published studies[2,3] revealed a sample size of 3 horses from each of grades B and C exercising laryngeal function would be necessary for the study to have 80% power. Client-owned horses with naturally occurring RLN were recruited into the study. Informed client consent was obtained from all owners.

Pre-operatively, exercising over-ground endoscopy was performed in all horses using a Videomed Overground Scope[a] to confirm the diagnosis of RLN (grade B or C exercising laryngeal function plus vocal fold collapse (VFC) +/- right medial deviation of the ary-epiglottic fold (MDAF), and to exclude horses which had other forms of dynamic upper respiratory collapse. The exercise test was tailored to the horses' usual mode of work, and the rider wore a GPS watch to record the speed and duration of exercise. A recording of respiratory noise was made during the test with a unidirectional cardiodid microphone (E608, Sennheiser [b]), which was attached to the endoscope using Velcro straps, and positioned at the right nostril. The microphone was connected to a digital recorder (DR40 handheld 4-track recorder, Tascam [c]) which was placed in the saddle-pad of the over-ground endoscope.

Laser surgery:

Horses were pre-medicated with flunixin meglumine (1.1 mg/kg IV, Flunixin Injection, Norbrook [d]), butorphanol (0.1 mg/kg IV, Butador, Chanelle [e]), and procaine benzyl penicillin (12 mg/kg IM, Depocillin, MSD animal health [f]). Animals were then sedated with romifidine (0.08 mg/kg IV, Sedivet, Boehringer Ingleheim Vetmedica [g]). Xylazine (0.4 mg/kg IV, Virbaxyl, Virbac [h]) was used to provide additional sedation if required.

Sedated horses were positioned in stocks and a left sided VeC was performed with a diode laser (VetArt 980 Diodenlaser[i]) under video-endoscopic guidance. The caudo-medial wall of the left ventricle was first grasped and the mucosa of the ventricle partially everted using 60cm long Equine Laryngeal Forceps [j] before the everted portion was excised using the diode laser with a power setting of 15W (continuous wave). The laryngeal forceps were then repositioned on the mid-section of the medial edge of the left vocal cord and the cord was excised using the laser at the same setting, using the method described by Henderson *et al.*[5]. Briefly, 2 horizontal cuts were made to transect the dorsal and ventral attachments of the vocal cord. A vertical cut was then made from dorsal to ventral to free the cord from it’s attachment to the lateral larynx. After the cord was excised, the laser surgery site was sprayed topically via a trans-endoscopic catheter with approximately 7ml of 2mg/ml dexamethasone solution (Dexadreson, Dechra[k]). Total laser energy used was not recorded as the laser unit utilized in this study did not generate this information.

Horses were discharged from the hospital an hour or so after surgery, when they had sufficiently recovered from sedation to travel. They were starved for 4 hours after surgery to allow the effects of topical local anaesthetic to wear off. A 10 day course of phenylbutazone (2 mg/kg BID PO for 5 days then 2mg/kg SID PO for 5 days, Equipalazone[k]) was prescribed. Topical 'throat spray' was not administered. Owners were instructed to box rest horses for the first week and then to turn out in a small paddock for 3 weeks before re-commencing normal ridden work. Owners were also asked to return the horse at 6-8 weeks post-operatively for a re-evaluation.

At the post-operative evaluation, exercising over-ground endoscopy and respiratory noise recordings were repeated in the same manner as pre-operatively.

Analysis of exercising endoscopy videos:

Videos were assigned a random number and were analysed in a blinded manner by a single ECVS diplomate with significant experience in interpreting exercising endoscopy. The presence of standard dynamic upper airway abnormalities[6] was recorded. Additionally, for each video, the left arytenoid cartilage was graded as stable, mildly unstably or markedly unstable[7] when the horse was exercising maximally.

Three freeze frames of each video were obtained from both pre- and post-operative endoscopic examinations, taken at a time when the respiratory obstruction was deemed to be maximal. From these, left-to-right quotient angle ratio (LRQ) and rima glottidis area ratio (RGA) (Figure 1) were calculated as described previously by Leutton and Lumsden[8] using image analysis software (Image J[l]). The mean values from the 3 freeze framed images were calculated for each horse pre- and post-operatively. Descriptive analysis of this data was described. Further statistical analysis of LRQ and RGA values is not presented as no power calculation had been performed for this data.

Respiratory sound analysis

From each pre- and post-operative sound recording, a 10 second section of respiratory noise was taken when the horse was exercising at maximal effort, near to the end of the exercise test. A semi-automated approach to formant identification and measurement was adopted and implemented in Matlab[m]. This provided a consistent basis for making comparisons between cases and more details of this methodology are provided in Supplementary Item S1. All recordings were high-pass filtered (using a finite impulse response filter of order 50) with a lower cut-off frequency of 200 Hz, to reduce the effects of low frequency ‘rumble’ due to hoof noise.

Inspiratory breaths were identified using a semi-automated approach (see S1). The duration of inspiration that was used for analysis was taken as being from 0.1 seconds before to 0.1 seconds after the peak of energy within each inspiration. Formants were identified using Linear Protective Coding analysis (see S1), with the search for formant peaks constrained to the following previously described[9] frequency regions: F1 = 0 - 600 Hz, F2= 900 - 2400 Hz and F3= 2800 - 4800 Hz. Within each of these regions the centre of the frequency analysis bin with highest local maximum energy was taken as being the centre of the formant. To measure the sound energy within each formant, a 2048-order third-octave filter centred on the formant’s central frequency was derived. The total amount of energy within the third-octave bands for each of ten inspiratory breaths was then summed. The relative total energy (dB) for each formant (F1, F2, F3) for each case was calculated as: 10 x $\log_{10}$ [summed energy from 10 inspirations].

Statistical analyses were performed using Minitab. Pre- and post-operative total sound energy values (dB) for each formant were compared using Wilcoxon Sign ranked test. Mann Whitney tests were used to compare between grade B and C horses for pre-operative sound energy levels, post-operative sound energy levels and reduction in sound energy levels. Statistical significance was set at $P \leq 0.05$.

Results:

Ten horses were included in the study; mean age was 6 years (range 3-15 years). Their use was: show-jumping (4), hunting (3), dressage (1), eventing (1), and National Hunt racing (1). From GPS data, the maximal speed attained (mean 19.7 km/h) and total distance cantered over (mean 1.6 km) were very similar for pre- and post-operative exercise tests for 9 horses that were cantered either in a ménage or in a field. One racehorse was only cantered at 18.6 km/h pre-operatively as it was not deemed fit enough to gallop, but post-operatively it was galloped at 38 km/h.

Pre-operatively, at rest, 1 horse had grade 3.1 laryngeal function, 5 had grade 3.2, 2 had grade 3.3 and 2 had grade 4 laryngeal function[1]. At exercise, 3 had grade B and 7 had grade C laryngeal function. All grade B horses had stable left arytenoid cartilages and only left sided VFC. Of the 7 grade C horses, 4 left arytenoid cartilages were deemed to be markedly unstable, 2 were mildly unstable and 1 was deemed to be stable. Six of 7 grade C horses had bilateral VFC, and 5/7 had mild right-sided MDAF (figure 2).

Surgery was completed without peri-operative complications in all cases. Horses were re-presented for follow-up examination at a mean of 8.5 weeks post laser surgery (range 6-16 weeks).

Post-operative endoscopic findings (Supplementary items 2-4):

Nine of 10 laser surgery sites had healed fully (figure 3) at the time of re-examination. One horse that represented at 6 weeks post-operatively had a roughened edge to the laser surgery site caused by small granuloma formation (figure 3). These granulomas were removed under sedation with a trans-endoscopic diode laser (10W continuous wave) and the horse discharged with a further 7 day course of phenylbutazone (2 mg/kg BID PO for 3 days then 2mg/kg SID PO for 4 days, Equipalazone[k]). This horse was re-examined 3 weeks later when the site appeared smooth (figure 3) and the post-operative exercise test, endoscopy and sound recording were performed at this time.

Post-operative exercising endoscopy (supplementary items 2-4) revealed ongoing right VFC (figure 4) in 6/7 grade C horses, and one grade B horse also showed mild right VFC. All 5 grade C horses that had mild right-sided MDAF pre-operatively continued to exhibit the same degree of MDAF post-operatively. Arytenoid cartilage stability at exercise appeared, subjectively, to be partially improved (from severely unstable to mildly unstable) after unilateral laser VC in 3 grade C horses (Supplementary item 4).

A summary of LRQ and RGA data is shown in Table 2. As would be expected, horses with grade B laryngeal function had larger LRQs and RGA ratios than those horses with grade C laryngeal function both pre- and post-operatively. Although statistical tests were not performed on these endoscopically generated data, for all horses, the mean pre- and post-operative LRQs were similar (0.58 +/- 0.19 pre-op vs 0.57+/- 0.19 post-op), indicating that the degree of arytenoid abduction was similar before and after surgery. The post-operative RGA ratios for all horses (mean = 0.28 +/- 0.10) were consistently larger than their pre-operative RGA ratios (mean = 0.24 +/-0.11).

Sound analysis:

Subjective description of pre-operative spectrograms:

All 3 Grade B horses had quite a different appearance of their spectrograms in the F2 formant frequency range compared to the 7 grade C horses. In grade B horses, the energy in F2 was generally less intense, and the band of high intensity 'abnormal' sound was confined to a much narrower frequency range within F2 (figure 5). This band of sound was approximately 500 Hz wide (range 450-550 Hz) and was contained within the upper half of F2 in all horses (centred at a mean of 1970 Hz, range 1725-2200 Hz). An abnormal band of sound could not be identified in the previously defined F3 formant frequency range (2800-4800 Hz) in any grade B horse (figure 5).

In grade C horses pre-operatively, inspiration was frequently louder than expiration, and the band of 'abnormal' inspiratory sound energy was spread right across the frequency range of F2 (900 - 2400 Hz, figure 6). In 3/7 grade C horses, all of which were graded as having markedly unstable left arytenoid cartilages, there was visibly increased sound energy in the F3 formant frequency range (2800-4800 Hz, figure 6). However in 4/7 grade C horses, 3 of which had mildly unstable arytenoids and 1 of which had a markedly unstable arytenoid, there was only very mild or no visibly increased sound energy in the F3 formant range. Although 6/7 grade C horses had MDAF, this could not be identified specifically within the spectrograms, probably because the whole of the F2 formants of these horses contained high levels of energy.

Objective analysis of audio files:

Pre-operatively, grade B horses (mean 23.9dB, range 17.5-29.9dB) had lower sound energy values in the F2 formant of inspiration than grade C horses (mean 31.6dB, range 27.8-34.3dB), but this finding was not statistically significant (P=0.07). Post-operatively there was also no significant difference (p=0.25) in sound energy values within the F2 formant of inspiration between grade B (mean 16.9 dB, range 15.1-18.1dB) and C (mean 22.7dB, range 14.2-33.3) horses.

The mean post-operative reduction in the energy within the 1/3rd octave band in F2 was -8.3dB (range -0.3- -13.3, SD 4.3), in F1 was 1.08 dB (range +18 - -8.9, SD 7.8), and in F3 was -2.3 (range +3.7 - -8.42, SD 4.1). Only the reduction in sound intensity of F2 was found to be statistically significant (P=0.05). Reduction in F2 sound intensity was not significantly different between horses with grade B or grade C exercising laryngeal function (P=0.27).

Discussion:

Unilateral VeC is one of the most common upper respiratory tract (URT) surgeries performed on performance horses, yet there is limited evidence available in the veterinary literature regarding this surgical technique. The major aims of VeC surgery are to reduce respiratory noise at exercise and improve ventilatory parameters in horses with RLN and VFC, however it is the absence of noise that is most commonly judged by owners, trainers and veterinary surgeons as the major measurement of 'success' following URT surgery.

Laser VeC is a minimally invasive procedure that has gained widespread popularity. It is commonly performed unilaterally on the left vocal fold either with or without concurrent laryngoplasty and is often performed with right sided ventriculectomy. No previous studies have objectively analysed unilateral laser VeC for treatment of vocal fold collapse in horses with naturally occurring RLN. Two previous studies performed in horses with experimentally induced grade 4 RLN have found that bilateral VeC (performed with a scalpel) or unilateral left laser VeC both restore sound levels to close to baseline (pre-neurectomy levels) in grade 4 horses[2,4]. This finding is rather surprising, given that all grade 4 horses can be assumed to have grade C laryngeal function[10,11], and in such horses, the left arytenoid is presumably still collapsing during inspiration post-VeC, causing ongoing respiratory obstruction and turbulent airflow. It has been suggested that the VeC procedure may, in some way, stabilize a previously unstable left arytenoid cartilage thus reducing the noise[3], but this theory has not been supported with endoscopic evidence. A clinical study of grade 4 RLN affected draft horses found that bilateral surgical VeC significantly reduced inspiratory noise, but these horses were only exercised at a trot (mean speed 4.6m/s)[4]. It would be fair to say that horses that are exercised at the canter and gallop are likely generate greater trans-tracheal negative pressures and therefore experience more severe degrees of arytenoid collapse than those exercised at a trot.

In naturally occurring cases of RLN, which are presented for surgery, a spectrum of laryngeal dysfunction can be observed, including ipsilateral VFC in conjunction with varying severities of arytenoid collapse. Additionally, horses with naturally occurring RLN appear to have a high prevalence of MDAF and right VFC. We do not know if these abnormalities occur in horses with experimentally induced RLN, but we assume that they might not. All published studies thus far that have objectively analysed sound production in horses with RLN have only included Havemeyer grade 4 (exercising grade C) horses[2-4,9], and this study is the first to perform sound analysis in horses with a range of resting (grade 3.1, 3.2, 3.3 and 4) and exercising (grades B and C) laryngeal function.

Equipment set up:

In the present study the microphone was placed close to the right nostril, similar to the method previously reported by Derksen *et al*[9]. In contrast to Derksen *et al.*'s[9] methodology where the microphone was attached to a cavesson noseband, we attached the microphone to the insertion tube of the over-ground endoscope, with the microphone positioned at the level of the right nostril. To assess if the presence of the scope had an effect on sound recordings, we performed a pilot study recording respiratory noise in 2 horses which were exercised with the scope in place, and then again without the scope in place but with the microphone attached via the bridle in a similar position. There was no discernible effect of the insertion tube of the endoscope being in place when sound recordings were made.

Additionally, in the current study, both pre- and post-operative recordings were made with the endoscope in place, so the presence of the endoscope should not have had any effect on comparison of spectrograms and objective measurements of sound.

Timing of re-evaluations

Although owners were asked to return their horses 6-8 weeks after surgery, there was some variation in timing of re-evaluation (mean 8.5 weeks, range 6-16 weeks) which is an unfortunate consequence of conducting a study in client-owned horses. Horses that were examined after a longer interval included the horse that had a second laser surgery at 6 weeks post-operatively, and several which were put out to grass for a prolonged rest period after surgery, thus were not fit enough to perform the exercise test at the designated 6-8 weeks. Previous studies have shown that the post-operative time period can have a variable effect on sound production between 60 and 120 days after VeC[2,3] and this may have had a small effect on our results.

Videoendoscopic findings

In this study 6/7 horses with grade C laryngeal function had bilateral VFC recognised when exercising endoscopic videos were carefully analysed. Bilateral VFC has also been reported in 35/35 of horses with naturally occurring RLN that subsequently underwent laryngoplasty [8]. Although some surgeons must have suspected this for some time, because they routinely advocate bilateral VeC[2,12], it is still common to only remove the left vocal fold in horses with RLN[13-15]. The current study and that of Leutton and Lumsden[8] suggest that bilateral vocal fold collapse is very common in horses with RLN. Equine surgeons may be wary of performing bilateral VeC because of the perceived risk of inducing ventral laryngeal webbing and stenosis, which can occur whether using a scalpel blade or a laser [16]. Aggressive bilateral VeC in association with laryngoplasty may also predispose horses to post-operative food aspiration and coughing (N. Ducharme, personal communication). Safer alternatives to full bilateral VeC include using a scalpel rather than a laser, leaving the ventral 5mm of each vocal cord in situ and therefore preserving the vocal fold fornix, suturing the edge of the fold to the axial border of the ventricle[15], only removing the dorsal half of the right vocal fold, or performing a right vocal cordotomy (rather than cordectomy) to induce scarring in the right vocal fold and thus reduce the severity of right VFC (F. Rossignol, personal communication).

Right sided MDAF has been previously reported in horses with RLN both pre- [8] and post-laryngoplasty [8,17,18]. If present pre-operatively, it should be addressed at the time of surgery. In our group of horses, the severity of right sided MDAF was mild in all cases, both pre- and post-operatively. The contribution of this minor degree of MDAF to respiratory obstruction is likely to be quite small, and its contribution to abnormal respiratory noise is unknown as there are no publications describing sound analysis of horses with MDAF. Based on previously published studies, its relatively high prevalence post-operatively after LP [8,17,18] might even lend weight to the practice of routine removal of the right ary-epiglottic fold in all horses undergoing laryngoplasty surgery.

The degree of left arytenoid abduction did not appear to be much changed after left laser VeC, as evidenced by very similar pre- and post-operative LRQs. Rima glottis area was slightly increased by the surgery in almost all horses, with grade C horses tending to have a larger increase, presumably because the left VFC was more obstructive in these cases compared to grade B horses. The small number of horses in this study should be considered when evaluating such small changes in endoscopic measurements. Subjectively, laser VeC appeared to partially improve the stability of the left arytenoid in 3/7 grade C horses that pre-operatively had marked instability of this structure, and these results support the theory first postulated by Robinson *et al*.[3].

Sound analysis

A semi-automated method of sound analysis was developed for this study, where the total energy contained within a 1/3 octave band centred around the peak of energy within F2 was calculated. We believe that this semi-automated method should be more accurate than previously reported methods which relied on visual inspection of the spectrogram to detect a single 'peak' of energy within each formant[2-4]. It is possible that evaluating the energy in the entire F2 frequency range (900-2400Hz) might better detect differences between grade B and C horses, and horses with stable and unstable arytenoid cartilages, rather than restricting evaluation to the energy within a 1/3 octave band.

Previous studies have analysed the sound spectrum of horses with experimentally induced (grade 4/4) RLN[2,3,9], which would all be expected to have grade C laryngeal function at exercise[10,11], but spectral analysis of grade B horses with vocal fold collapse has not previously been reported. Only 3 grade B horses were included in this study, but subjectively, visual analysis of their spectrograms demonstrated a much narrower abnormal band (approximately 500 Hz wide) of sound energy observed during inspiration, compared with the broad band of increased energy within F2 seen in horses with grade C laryngeal function. Certainly, horses with a stable, partially abducted arytenoid and vocal fold collapse make a higher pitched inspiratory noise often described as a ‘whistle’, whereas those with complete collapse of the arytenoid are often described as making a louder, lower pitched inspiratory ‘roar’. In this study it would appear that the spectrograms reflect this difference in audible abnormal sound in clinical cases. Additionally, abnormal sound energy could not be visually identified in the F3 formant in grade B horses.

It has recently been proposed that an additional grade, ‘D’, of exercising laryngeal function should be introduced to differentiate between a minimally abducted but relatively stable left arytenoid and one that dynamically collapses into the contralateral rima glottidis during inspiration[19]. If the current study had included a larger number of horses, we believe it is likely that it would be possible to make a differentiation between grades C and D using sound analysis, as the ‘noisiest’ spectrograms and audio files were clearly from horses which had markedly unstable grade C (the proposed grade ‘D’) arytenoids. Three out of 4 grade C horses with markedly unstable arytenoids were the only horses that had visibly increased sound energy within the F3 formant. We therefore suggest that increased sound energy within the F3 formant may be associated with active collapse of the arytenoid cartilage as is seen in the proposed grade D horses. It is likely that the absence of abnormal sound energy in the F3 formant in 7/10 horses in this study (pre-operatively) would explain why no statistically significant difference was found when comparing F3 sound levels pre- and post-operatively.

Although the energy in the F2 formant was statistically significantly reduced after laser VeC when the group of 10 horses was analysed, a good proportion of horses (particularly unstable grade C horses) still made an audible abnormal inspiratory noise after surgery (supplementary items 3 and 4). This illustrates that just because a clinical measurement has statistically significantly improved, a clinically obvious abnormality may still be present. Continuing abnormal respiratory noise after VeC was not caused by incomplete resection of the fold and collapse of left vocal fold remnants, but was more likely attributable to other, often pre-existing, dynamic obstructions including continuing arytenoid instability in grade C horses, and/or right VFC and MDAF. The F2 formant has been identified as the formant that most closely reflects noise associated with collapse of the left vocal cord, ventricle and corniculate process and body of the arytenoid cartilage[2,3,4]. However, it is not known which frequency ranges abnormal noise created by other collapsing structures (such as MDAF) would lie within and experimental models to evaluate these do not exist, to our knowledge. This would be an interesting area of further research.

Conclusions

This study was limited by its small sample size and the fact that multiple dynamic disorders made sound analysis challenging. It has, however, highlighted several findings that are of clinical relevance to horses with RLN: firstly, that in horses with Grade C laryngeal function, bilateral VFC and right sided MDAF are extremely common. Secondly, that horses with grade B laryngeal function and VFC make significantly less noise and have a narrower band of abnormal energy in the F2 formant as compared to horses with grade C laryngeal function and arytenoid cartilage collapse. Thirdly, this study also suggests that in some cases, laser VeC can stabilise a previously unstable arytenoid cartilage to some degree. Finally, due to continued right VFC, right MDAF and most importantly, continuing arytenoid instability, unilateral laser VeC is not necessarily a useful treatment option for horses with unstable grade C laryngeal function at exercise. This is especially true if the clinical resolution of respiratory sound is the main objective of surgery. Bilateral VeC or laryngoplasty plus VeC +/- right ary-epiglottic fold resection may be a better option for horses with grade C RLN.

Manufacturers' addresses

[a] Videomed GmbH , Munich, Germany

[b] Sennheiser UK Ltd., Marlow, UK

[c] TEAC UK Ltd, Guildford, UK

[d] Norbrook, Corby, UK

[e] Chanelle UK, Hungerford, UK

[f] MSD animal health, Milton Keynes, UK

[g] Boehringer Ingelheim Vetmedica, Bracknell, UK

[h] Virbac, Bury St Edmunds, UK

[i] Jørgen Kruuse A/S, Langeskov, Denmark

[j] Karl storz GmbH & Co. KG, Tuttlingen, Germany

[k] Dechra Veterinary Products, Shewsbury, UK

[L] National Institutes of Health, Bethesda, Maryland, USA.

[m] MathWorks, Massachusetts, USA

References

1. Robinson, N.E. (2004) Consensus statements on equine recurrent laryngeal neuropathy: conclusions of the Havemeyer Workshop. September 2003, Stratford upon Avon. Equine vet. Educ. 16, 333-336.

2. Brown, J.A., Derksen, F.J., Stick, J.A., Hartmann, W.M. and Robinson, N.E. (2003) Ventriculocordectomy reduces respiratory noise in horses with laryngeal hemiplegia. Equine vet. J. 35, 570-574.

3. Robinson, P., Derksen, F.J., Stick, J.A., Sullins, K.E., DeTolve, P.G. and Robinson, N.E. (2006) Effects of unilateral laser-assisted ventriculocordectomy in horses with laryngeal hemiplegia. Equine vet. J. 38, 491-496.

4. Cramp, P., Derksen, F.J.D., Stick, J.A., Nickels, F.A., Brown, K.E., Robinson, P. and Robinson, N.E. (2009) Effect of ventriculectomy versus ventriculocordectomy on upper airway noise in draft horses with recurrent laryngeal neuropathy. Equine vet. J. 41, 729-734.

5. Henderson, C.E., Sullins K.E., Brown J.A. (2007) Transendoscopic, laser-assisted ventriculocordectomy for treatment of left laryngeal hemiplegia in horses: 22 cases (1999-2005).J Am Vet Med Assoc. 231,1868-72.

6. Barnett, T.P., Smith, L.C., Cheetham, J., Barakzai, S.Z., Southwood, L., Marr, C.M. (2015) A call for consensus on upper airway terminology. Equine Vet J.47, 505-7

7. Barnett, T.P., O'Leary J.M., Parkin, T.D.H., Dixon, P.M., Barakzai, S.Z. (2013) Long-term maintenance of arytenoid cartilage abduction and stability during exercise after laryngoplasty in 33 horses. Vet Surg. 42, 291-5

8. Leutton, J.L. and Lumsden, J.M. (2015) Dynamic respiratory endoscopic findings pre and post-laryngoplasty in Thoroughbreds. Equine vet. J. 47, 531-6

9. Derksen, F.J., Holcombe, S.J., Hartmann, W., Robinson, N.E., Stick, J.A.(2001) Spectrum analysis of respiratory sounds in exercising horses with experimentally induced laryngeal hemiplegia or dorsal displacement of the soft palate. Am J Vet Res. 62,659-64
10. Lane J.G., Bladon, B., Little, D.R., Naylor, J.R., Franklin, S.H. (2006) Dynamic obstructions of the equine upper respiratory tract. Part 2: comparison of endoscopic findings at rest and during high-speed treadmill exercise of 600 Thoroughbred racehorses. Equine Vet J. 38, 401-7.
11. Barakzai, S.Z., and Dixon, P.M. (2011) Correlation of resting and exercising endoscopic findings for horses with dynamic laryngeal collapse and palatal dysfunction. Equine Vet J. 43,18-23
12. Fulton, I.C., Anderson, B.A., Stick, J.A., Robertson, J.T. (2012). Larynx. In: Equine Surgery. Eds. Auer, J. and Stick, J.A. Pub. Elservier, St Louis, Missouri. Pp 592-623
13. Taylor, S.E., Barakzai, S.Z., Dixon, P.M. (2006) Ventriculocordectomy as the sole treatment of recurrent laryngeal neuropathy: Long term results from 98 clinical cases in a mixed breed population of horses. Vet. Surg. 35, 653-657
14. Kraus, B.M., Parente, E.J., Tulleners, E.P. (2003) Laryngoplasty with ventriculectomy or ventriculocordectomy in 104 draft horses (1992-2000). Vet Surg.32, 530-8.
15. Witte, T.H., Mohammed, H.O., Radcliffe, C.H., Hackett, R.P., Ducharme, N.G. (2009) Racing performance after combined prosthetic laryngoplasty and ipsilateral ventriculocordectomy or partial arytenoidectomy: 135 Thoroughbred racehorses competing at less than 2400 m (1997-2007). Equine Vet J. 41: 70-5

16. Davidson, E.J., Martin, B.B., Rieger, R.H., Parente E.J. (2009) Exercising videoendoscopic evaluation of 45 horses with respiratory noise and/or poor performance after laryngoplasty. Vet. Surg 39, 942-948.

17. Kane-Smyth ,J., Barnett, T.P., O'Leary, J.M., Dixon, P.M.(2016) Surgical Treatment of Iatrogenic Ventral Glottic Stenosis Using a Mucosal Flap Technique. Vet Surg. 45, 436-42 1.

18. Barnett, T.P., Dixon, P.M., Parkin, T.D.H. and Barakzai, S.Z. (2011) Long-term exercising video-endoscopic examination of the upper airway following laryngoplasty surgery: A prospective cross-sectional study of 41 horses. Equine Vet J. 45,,593

19. Rossignol, F., Brandenberger, O., Perkins, J., Mespoulhes-Rivière, C., Marie, JP., Ducharme, N. (2016) Modified first cervical nerve transplantation technique for the treatment of recurrent laryngeal neuropathy in horses. ACVS conference, Seattle.

Supporting information:

Supplementary item 1: Detailed methodology of sound analysis.

Supplementary item 2: grade B horse pre and post-op

Supplementary item 3 : grade C pre- and post op with severely unstable arytenoid both pre- and post-op

Supplementary item 4: grade C pre- and post-op with severely unstable arytenoid pre-op which is partially stabilised post-op.